

WE CLAIM:

1. A dual-purpose primer for amplifying a target nucleotide sequence in a target molecule, wherein the target nucleotide sequence contains a site of interest proximal to or contained within a secondary structure forming region that, in the absence of the primer, results in an unwanted secondary structure in an amplicon formed under amplification conditions so as to prevent detection of the site of interest, wherein the primer comprises: (a) a primer sequence complementary to a segment of the target nucleotide sequence other than the secondary structure forming region; and (b) a blocking sequence substantially complementary to a segment of the secondary structure forming region to prevent formation of the unwanted secondary structure.
2. The primer of claim 1, wherein the site of interest is a nucleic acid sequence.
3. The primer of claim 2, wherein the site of interest is a single nucleotide polymorphism.
4. The primer of claim 1, wherein the primer sequence is complementary to one terminus of the target molecule containing the target nucleotide sequence.
5. The primer of claim 1, further including a nonhybridizing spacer between the primer sequence and the blocking sequence.
6. The primer of claim 5, wherein the spacer is non-nucleotidic.
7. The primer of claim 6, wherein the spacer is comprised of a synthetic hydrophilic oligomer.
8. The primer of claim 7, wherein the spacer is comprised of about 3 to about 50 alkylene oxide units selected from ethylene oxide and combinations of ethylene oxide and propylene oxide.
9. The primer of claim 5, wherein the spacer is nucleotidic.
10. The primer of claim 9, wherein the spacer is comprised of a sequence of non-natural nucleotides.

11. The primer of claim 10, wherein the non-natural nucleotides are selected from iso-guanine and iso-cytosine.

12. The primer of claim 9, wherein the spacer is an oligomeric segment comprised of a recurring single nucleotide.

13. The primer of claim 9, wherein the probe sequence and the spacer are separated from each other by a means for halting transcription therebetween.

14. The primer of claim 13, wherein the means for halting transcription is an arresting linker.

15. The primer of claim 14, wherein the arresting linker comprises at least one modified nucleoside.

16. The primer of claim 15, wherein the modified nucleoside is an N⁴-modified pyrimidine.

17. The primer of claim 1, further comprising a detectable label.

18. The primer of claim 17, wherein the detectable label is selected from the group consisting of fluorescers, chemilumescers, dyes, biotin, haptens, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, enzyme subunits, metal ions, electron-dense reagents, and radioactive isotopes.

19. A method for amplifying a target nucleotide sequence in a target molecule, wherein the target nucleotide sequence contains a site of interest proximal to or contained within a secondary structure forming region capable of forming an unwanted secondary structure in an amplicon formed under amplification conditions, comprising:

contacting the target nucleotide sequence under hybridizing conditions, together or sequentially, with a dual-purpose primer according to claim 1 complementary to one terminus of a first strand of the target molecule, a second primer complementary to the opposing terminus of the second strand of the target molecule, nucleotides appropriate to said amplification, and an agent for polymerization of the

nucleotides, wherein amplicons formed during said method do not contain the unwanted secondary structure, such that the site of interest is accessible to a hybridizing oligonucleotide.

20. The method of claim 19, wherein the agent for polymerization is a DNA polymerase.

21. The method of claim 19, wherein the agent for polymerization is a DNA ligase.

22. The method of claim 19, wherein the agent for polymerization is an RNA polymerase.

23. The method of claim 19, wherein the agent for polymerization is an RNA reverse transcriptase.

24. In a method for conducting the polymerase chain reaction (PCR) to amplify a sequence of a double-stranded target DNA molecule having a first terminus and a second terminus, which comprises (a) heating a sample containing the double-stranded DNA to a temperature effective to denature the DNA and thereby provide a first single strand of DNA and a second strand of DNA, (b) contacting the denatured DNA with first and second oligonucleotide primers each comprised of a target binding sequence complementary to the first terminus of the first DNA strand and to the second terminus of the second DNA strand, respectively, (c) cooling the sample so as to allow hybridization of first and second oligonucleotide primers to the first and second strands of DNA, respectively, (d) replicating the DNA using a DNA polymerase, and repeating the aforementioned steps (a) through (d) to provide multiple copies of the sequence of double-stranded DNA, the improvement comprising employing as the first primer a dual-purpose primer according to claim 1.

25. The primer of claim 24, wherein the detectable label is selected from the group consisting of fluorescers, chemilumescers, dyes, biotin, haptens, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, enzyme subunits, metal ions, electron-dense reagents, and radioactive isotopes.

26. An amplicon formed by the action of a DNA polymerase on the primer of claim 1 hybridized to the target nucleotide sequence.

27. A kit for determining the genotype of an individual, comprising a dual-purpose primer according to claim 1, nucleotides appropriate to amplification of an oligonucleotide sequence, and an agent for polymerization of the nucleotides.

28. A kit for determining the genotype of an individual, comprising a dual-purpose primer according to claim 1, a second primer, nucleotides appropriate to DNA amplification, an agent for polymerization of the nucleotides, an allele specific hybridization (ASH) probe having a nucleotide capture region, and color-coded detecting means having a nucleotide capture region complementary to the nucleotide capture region on said ASH probe,

wherein the nucleotide capture region on said detecting means is complementary to said ASH probe such that the target nucleotide sequence is identified by the color-coding of said detecting means.

29. The kit of claim 28, wherein the detecting means is a multiplex detecting means.

30. The kit of claim 29, wherein the multiplex detecting means comprises a detectable solid substrate.

31. The kit of claim 30, wherein the detectable solid substrate is a detectable microsphere.

32. A hybridization probe comprising (a) a probe nucleotide sequence complementary to a first nucleotide sequence in a target molecule, and (b) a blocking sequence substantially complementary to a second nucleotide sequence in a target molecule, wherein hybridization of the blocking sequence with the second nucleotide sequence prevents secondary structure formation in the second nucleotide sequence that would otherwise interfere with hybridization of the probe sequence to the first nucleotide sequence.

33. The hybridization probe of claim 32, further comprising a detectable label.

34. The hybridization probe of claim 33, wherein the detectable label is selected from the group consisting of chemiluminescent labels, fluorescent labels, radioactive labels, multimeric DNA labels, dyes, enzymes, enzyme modulators, detectable solid substrates, and metal ions.

35. A method of performing a hybridization assay for detecting the presence of a target nucleotide sequence in a target molecule, wherein the target nucleotide sequence is proximal to or contained within a secondary structure forming region capable of forming an unwanted secondary structure that would prevent detection of the target nucleotide sequence, the method comprising:

contacting the target molecule under hybridizing conditions with the hybridization probe of claim 33, such that hybridization of the probe to the target molecule prevents formation of the unwanted secondary structure and allows detection of the target nucleotide sequence.

36. The method of claim 35, wherein the target molecule is obtained from a human individual.

37. The method of claim 35, wherein the target molecule is bacterial in origin.

38. The method of claim 35, wherein the target molecule is viral in origin.

39. The method of claim 38, wherein hybridization of the first hybridization probe sequence with the target nucleotide sequence is diagnostic of a disease caused by the virus.